Validation of river typologies using molecular information from diatoms

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Abstract

The European Water Framework Directive (2000/60/EC) includes biological assessment of water bodies that has been implemented for many years. Indicator organisms such as diatoms respond to geological and hydrological features of rivers by modifying their structure. Therefore, when implementing the WFD, it was necessary to establish type-specific reference conditions to be able to measure the deviations of sampled communities due to anthropogenic impact. HTS-related eDNA metabarcoding has been developed to complement or even replace traditional approaches for its rapid, low-cost and highly accurate identification of communities for assessment of rivers’ ecological status (e.g. Mortágua et al., 2019; Pérez-Burillo et al. 2020) and proved to provide even more in-depth information about biological elements. The use of this information without assignment to species is being addressed once it eliminates the limiting factor of the reference database incompleteness and may provide new ecological information (e.g. Feio et al., 2020; Rivera et al., 2020).

Since WFD requires the establishment of reference conditions for each water body type, for eDNA methods’ implementation it will be essential to review, confirm or reformulate, and perhaps create new typologies.

Hereupon, the aim of this study is to analyze diatom communities from different typologies of Portuguese rivers resulting from DNA metabarcoding data and compare it with current typology system. To do so, we will verify the consistency of biological groups included in
each type, validate the molecular data, analyze the correspondence of OTU/ISU/ESV to environmental characteristics of rivers.

A total of 154 sampling sites were selected from central Portugal and northern Portugal in 2017 and 2019. The biofilm was collected for morphological identification and DNA sequencing of diatoms. Reference sites were selected for 4 river types (mountain, littoral, small and medium-large northern rivers) based on a set of pressure information (water quality, hydromorphology, land use and riparian zones). Diatom inventories were obtained from molecular and morphological analysis. DNA sequences were treated using Mothur software which processed two bioinformatic strategies in order to obtain the final ISU and OTU tables, while ESVs were treated with DADA2 package from R. Identification and counting of diatom valves took place under the light microscope concerning the morphological approach.

We expect results to validate the molecular data for each typology either when assigning to species or not, and to understand whether it is necessary to establish new typologies for future use of the molecular approach in ecological assessment of rivers.


**Keywords**

Diatoms; DNA metabarcoding; river types; WFD; bioassessment

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